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Dynamics of extracellular polymeric substance (EPS) production and loss in an estuarine, diatom-dominated, microalgal biofilm over a tidal emersion–immersion period

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Abstract

We studied patterns of production and loss of four different extracellular polymeric substance (EPS) fractions—colloidal carbohydrates, colloidal EPS (cEPS), hot water (HW)–extracted and hot bicarbonate (HB)–extracted fractions—and community profiles of active (RNA) bacterial communities by use of Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis of reverse transcription–polymerase chain reaction amplified 16S rRNA in mudflats in the Colne Estuary, United Kingdom, over two tidal emersion–immersion cycles. Colloidal carbohydrates and intracellular storage carbohydrate (HW) increased during tidal emersion and declined during tidal cover. The dynamics of cEPS and uronic acid content were closely coupled, as were the HB fraction and HB uronic acids. Changes in monosaccharide profiles of HW and HB fractions occurred during the diel period, with some similarity between cEPS and HB fractions. Increasing enzymatic release rates of reducing sugars and increased reducing sugar content were correlated with increased concentrations of colloidal carbohydrate and cEPS during the illuminated emersion period, and with the amount of HB-extracted uronic acids (the most refractory EPS fraction measured). Loss of reducing sugars was high, with sediment concentrations far below those predicted by the measured in situ release rates. T-RFLP analysis revealed no significant shifts in the overall taxonomic composition of the active bacterial community. However, 12 of the 59 terminal restriction fragments identified showed significant changes in relative abundance during the tidal cycle. Changes in the relative abundance of three particular terminal restriction fragments (bacterial taxa) were positively correlated to the rate of extracellular hydrolysis. Losses of chlorophyll *a* and colloidal and cEPS (up to 50–60%) occurred mainly in the first 30 min after tidal cover. About half of this may be owing to in situ degradation, with ''wash away'' into the water column accounting for the remainder.

Microphytobenthic biofilms in intertidal sediments play an important role in the ecology of estuarine systems (Underwood and Kromkamp 1999). The species composition of microphytobenthos (MPB) is diverse, with diatoms, euglenids, and cyanobacteria being the dominant groups of photoautotrophs. In fine-sediment intertidal habitats, the major group of organisms are motile pennate diatoms. Intertidal biofilms form at the surface of sediment during tidal exposure periods, with cells showing endogenous rhythms of vertical migration linked to tidal and diel cycles (Consalvey et al. 2004). During motility, benthic diatoms produce extracellular polymeric substances (EPSs), a complex mixture of polysaccharides and glycoproteins (Wustman et al. 1997; Chiovitti et al. 2003; Higgins et al. 2003). This EPS, combined with other extracellular carbohydrates (ranging from oligosaccharides through to polymers >100 kD) contribute to the mucilage component of the intertidal biofilms (de Brouwer et al. 2003; Stal 2003; Underwood and Paterson

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2003). These mucilages are important agents of sediment biostabilization (Underwood and Paterson 2003) and as a carbon source for heterotrophic organisms (Decho 1990; Middelburg et al. 2000).

Numerous studies have investigated the distribution and production rates of colloidal (water-soluble) carbohydrates in intertidal sediments (de Brouwer et al. 2003; Underwood and Paterson 2003 and references therein). In the first study of in situ rates of diatom EPS production, Smith and Underwood (1998) showed that the flux of organic carbon from photosynthesis to excretion was rapid (<1 h), but that ^{14}C-labelled EPS was produced up to 4 h after carbon assimilation in both the light and the dark. Colloidal carbohydrates are produced during daytime low-tide periods, with concentrations correlated significantly with biomass (Underwood and Smith 1998*a*) and related to rates of photosynthesis (de Brouwer and Stal 2001; Stal 2003). Studies of EPS production in diatom culture have revealed a complex set of pathways, EPS types, and responses to environmental conditions (Underwood and Paterson 2003; Magaletti et al. 2004; Underwood et al. 2004). More recently, additional extraction protocols (hot water, hot bicarbonate, hot alkali extractions) developed in culture studies (Wustman et al. 1997; Chiovitti et al. 2003, 2004) have been applied to estuarine diatom species and biofilms (Bellinger et al. 2005). These new extractions isolate additional EPS fractions that possess significantly different monosaccharide distributions and additional components (uronic acids, sulphate). These polymers may have significantly different structural properties within biofilms (Decho 2000; Stal 2003; Underwood and Paterson 2003).

Despite the importance of EPS in the functioning of MPB biofilms, there is a scarcity of information on the loss processes affecting EPS in intertidal sediments. EPS components may be removed into the overlying water column during high tide (either by physical erosion of surface sediment or by dissolution) (Underwood and Smith 1998*b*; Orvain et al. 2003), used directly by deposit-feeding invertebrates and vertebrates (Decho 2000) or microbial heterotrophs (Decho 2000; Middelburg et al. 2000; Hamels et al. 2004), or degraded by photolysis (Moran et al. 2000). The relative importance of each of these processes is likely to vary depending on the properties of individual EPS types.

The close physical proximity and high substrate concentrations within biofilms and other similar systems has been shown to promote a high degree of algal-bacterial coupling (Simon et al. 2002; Romaní et al. 2004). In intertidal sediments, bacterial and algal production are linked (van Duyl et al. 1999), especially in sediments with low organic content (Köster et al. 2005), and rapid use of low-molecular-weight compounds in particular has been recorded (van Duyl et al. 1999; Underwood 2002). A few studies have demonstrated extracellular enzyme activity related to algal activity in intertidal biofilms (Ruddy et al. 1998; van Duyl et al. 1999, 2000), but these have not specifically investigated the degradation of EPS. It is not known to what extent bacterial activity is tightly coupled to diatom EPS production or whether specific EPS-degrading bacteria occur. Complex polysaccharide molecules may be degraded at different rates (Arnosti 2000) and individual mono- and di-saccharides used preferentially by bacteria (Arnosti and Repeta 1994; Giroldo et al. 2003). With the development of molecular approaches to study the active bacterial community via reverse transcription–polymerase chain reaction (RT-PCR) amplification of 16S rRNA (Felske et al. 1996) coupled to fingerprinting analysis, for example, by Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis (Liu et al. 1997; Osborn et al. 2000), the response of bacterial communities to short-term environmental changes, such as tidal cycling and EPS production in sediments can now be investigated.

In the present study, we investigated the short-term changes in the concentrations of different EPS fractions in diatom-rich biofilms over tidal emersion–immersion–emersion cycles. We hypothesized that greater temporal changes in concentration and monosaccharide composition would be seen in the colloidal carbohydrate fractions with tidal exposure and cover compared with the more structural EPS fractions. The sampling regime was designed to sample biofilms immediately after tidal cover, to determine how quickly different EPS components were removed from the biofilm. We also hypothesized that there would be significant correlations among the composition of the active bacterial community, the concentrations of different EPS fractions, and hydrolytic enzyme activity within the biofilms.

Sampling and methods

Experimental overview—Surface sediment samples (top 2 mm) were collected from intertidal mudflats in the Colne Estuary at six time points over a tidal cycle; 13:00 h, 15:30 h, 17:30 h (during tidal emersion), 18:00 h, 19:00 h (30 min and 1.5 h after immersion, respectively), and 04:30 h (during next tidal emersion phase) on 27–28 May 2003 and 11–12 June 2003. All samples were extracted on site and frozen immediately for further analysis. Measurements concentrated on changes in concentrations of water-soluble (colloidal and colloidal EPS [cEPS]), bound hot water (HW)–extracted and hot-bicarbonate (HB)–extracted carbohydrate fractions, associated uronic acid content of these fractions, the monosaccharide composition of different carbohydrate fractions, liberation of reducing sugars as a measure of extracellular enzyme activity, and shifts in the composition of the active bacterial community over a tidal cycle, with emphasis on short-term temporal changes with tidal cover.

Sampling—Sampling took place on 27–28 May 2003 at Alresford Creek (51°50.2′N, 0°59.5′E), Essex, United Kingdom, situated midway along the Colne Estuary. Biofilms at Alresford Creek are abundant and diatom-dominated, with some euglenoids present (Underwood et al. 2005). The salinity gradient ranges from 18–28% and the sediment has a high proportion (80–95%) of clay particles. To allow comparison of sediment EPS dynamics over a duplicate tidal cycle, an identical set of measurements was made on 11–12 June 2003 on an intertidal mudflat at Wivenhoe (51°51.2′N, 0°58.0′E), Colne Estuary (~3 km from Alresford Creek). The mudflats at Wivenhoe have similar sediment and salinity characteristics to those in Alresford Creek (Dong et al. 2000).

At the beginning of each sampling regime, twelve 1-m^2

quadrats were randomly placed in an area (300 m^2) of dense biofilm at approximately mean tide level. Two quadrats were randomly designated to each sampling time. Within each quadrat, 10 cores (internal diameter, 7.5 cm; length, 15 cm) were removed from the sediment (total $n = 20$ for each time point). Each of these cores had the surface 2 mm of sediment removed for analysis of carbohydrate fractions, chlorophyll (Chl) *a*, and bacterial community composition by use of aluminum contact cores (Honeywill et al. 2002). Contact cores are filled with liquid nitrogen and directly freeze, by contact, the surface sediment within a 2-mm-deep recess on their underside. Frozen samples were returned to the laboratory. Overall, nine different carbohydrate fractions were measured: total, colloidal, cEPS, HW- and HB- extracted carbohydrates determined by the phenol-sulphuric acid assay (Dubois et al. 1956), and the uronic acid content of the colloidal (colloidal uronics), HW-extracted (HW uronic content) and HB-extracted (HB uronic content) fractions. Sediment Chl *a* measurements were paired with carbohydrate measurements.

To measure sugar release by extracellular enzyme activity, monosaccharide composition and nutrient pore water concentrations, nine additional sediment cores, three for each measurement (internal diameter, 7.5 cm; length, 15 cm; $n = 6$ overall) were extracted from each quadrat at each time point. The top 2 mm of surface sediment was removed and frozen (−20°C) on site.

Immediately preceding tidal cover, cores (internal diameter, 7.5 cm; length, 15 cm), each connected by a 2-m line to a plastic float, were placed with their upper rims flush into the sediment within the designated quadrats. Sampling was accomplished by snorkeling, using the lines to locate cores (visibility was zero owing to high turbidity). The sites were covered to a depth of 1.5 m of water by 19:00 h.

Sediment Chl a *analysis*—Sediment samples were taken for Chl *a* analysis at each time point by use of contact cores. All samples were frozen and stored at −20°C. Samples were freeze-dried and crushed. Four microliters of 100% methanol (saturated with $MgCO_3$) was added to 100 mg of each sediment sample, mixed, and incubated in the dark at 4°C for 24 h. Samples were mixed and centrifuged (3,000*g*, 15 min). Absorbance was measured at 665 nm and 750 nm (Lorenzen 1967). Samples were acidified and absorbance was determined at the above wavelengths to correct for phaeo-pigment content. Chl *a* was quantified (μg g^{-1} dry weight of sediment) by use of appropriate equations (Stal et al. 1984).

Sediment pore water nutrient analysis— Pore water was extracted by centrifugation (3,000*g*) from thawed surface sediment (top 2 mm), and filtered though glass-fiber filters (GF/C, Whatman). Nutrient concentrations (NO_3^-, NO_2^-, SiO_3^-, and PO_4^{3-}), were measured by use of a segmented flow autoanalyser (Skalar SAN^{plus}, Skalar Analytical) (Dong et al. 2000).

Sediment total carbohydrate analysis—Ten milligrams of freeze-dried sediment samples from each time point was placed into centrifuge tubes. One milliliter of dH_2O was added to each sample and mixed. Total carbohydrate content was measured by use of the phenol-sulphuric acid assay (Dubois et al. 1956); 5 mL of 0.5% phenol and 2.5 mL of concentrated H_2SO_4 were added to each sample and left to cool for 30 min. Samples were centrifuged (3,000*g* for 15 min), and supernatant was measured spectrophotometrically at 485 nm. H_2O blanks were treated as above. Total carbohydrate was quantified (μg g^{-1}, μg Chl a^{-1} dry weight sediment) as glucose equivalents.

Sediment water-soluble carbohydrate analysis—Colloidal carbohydrate content was measured by weighing 100 mg of freeze-dried sediment from each sampling point into centrifuge tubes. Four milliliters of saline (25%) was added and vortex-mixed. Samples were incubated for 30 min at 25°C. After centrifugation (3,000*g*, 15 min), 1 mL of supernatant was pipetted into boiling tubes, and colloidal carbohydrate content was measured using the phenol-sulphuric acid assay (see previous section). One milliliter of supernatant was retained for uronic acid quantification (see next section).

One and a half milliliters of the remaining supernatant from the colloidal fraction was added to 3.5 mL of ethanol (i.e., 70% v/v final concentration) to measure the cEPS fraction. Samples were covered and left for 24 h at 4°C to precipitate. Following centrifugation (3000 *g*, 15 min), supernatant was discarded and the cEPS pellets were resuspended in 1 mL of dH_2O and measured by use of the phenol sulphuric acid assay (Smith and Underwood 1998). Colloidal and cEPS carbohydrates were quantified (μg g^{-1}, μg Chl a^{-1} dry weight sediment) as glucose equivalents.

Sediment HW- and HB-extracted carbohydrate analysis—After extraction of the easily solubilized colloidal material, further carbohydrate fractions were extracted following procedures developed for diatom cells and sediments (Wustman et al. 1997; Bellinger et al. 2005). Sediment pellets were retained from the colloidal carbohydrate analysis (see above), and all supernatant was discarded. Four milliliters of ethanol was added to pellets to defat and remove pigments (ethanol fraction discarded). Four milliliters of dH_2O was added to each pellet, mixed, and incubated in a water bath (95°C, 1 h). After centrifugation (3,000*g*, 15 min), this HW soluble fraction was quantified for carbohydrates by use of the phenol-sulphuric acid assay and for uronic acids (see following).

Four milliliters of 0.5mol L^{-1} $NaHCO_3$ was added to the remaining pellet, mixed, and incubated in a water bath (95°C, 1 h). After centrifugation (3,000*g*, 15 min), the HB fraction was removed and quantified for carbohydrates by use of the phenol-sulphuric acid assay and for uronic acids. HW- and HB-extracted carbohydrates were quantified (μg g^{-1}, μg Chl a^{-1} dry weight sediment) as glucose equivalents.

Sediment uronic acid analysis—Aliquots of supernatant retained from the colloidal and HW- and HB-extracted carbohydrate analyses were used to determine uronic acid content by use of the carbazole method (Bellinger et al. 2005). One milliliter of supernatant from each of the above fractions were placed in 5 mL frozen (−80°C) sodium tetraborate in concentrated H_2SO_4 and kept on ice. Samples were vortex-mixed and incubated in a water bath at 100°C for 12

min. Samples were cooled on ice to room temperature (20°C), and 200 μL of carbazole reagent (1 g carbazole in 100 mL absolute ethanol) was added, mixed, and incubated at 100°C for 15 min. Uronic acid was measured spectrophotometrically at 520 nm. Uronic acid content was quantified as glucuronic acid equivalents. Measured uronic acids are a subfraction of the total carbohydrate concentration determined by the method of Dubois et al. (1956). In this article we use the term ''carbohydrate'' to define values obtained by use of the Dubois method (which gives a combined measure of hexoses, pentoses, and uronic acids) and the term ''uronic acids'' specifically for the component of the carbohydrate pool measured by the carbazole method.

Monosaccharide composition by gas chromatography/mass spectroscopy analysis—Monosaccharide determination was carried out on cEPS, HW, and HB carbohydrate fractions. From each quadrat, three large cores were collected (internal diameter, 7.5 cm), and the 2 mm of surface was freeze-dried. Four grams of dry sediment were sequentially extracted as above using 40 mL of 25% saline followed by ethanol (discarded), 30 mL of dH_2O, and 30 mL of 0.5 mol L^{-1} $NaHCO_3$ (95°C for 1 h for both the HW and HB fractions). cEPS was precipitated from the colloidal carbohydrate fraction in 70% ethanol (v/v); HW and HB soluble carbohydrates were dialyzed in a 10- to 12-kD filter against dH_2O, and all samples were freeze-dried for analysis.

Monosaccharide profiles were determined in triplicate (when applicable) for each fraction by use of the methods described by Wustman et al. (1997). Briefly, 1–5 mg of freeze-dried carbohydrate material was hydrolyzed in 2 mol L^{-1} triflouroacetic acid at 121°C for 3 h, followed by reduction to alditol acetates for identification/quantitation by gas chromatography/mass spectroscopy (Finnigan-MAT Magnum Ion Trap Gas Chromatography/Mass Spectroscopy). Identification and quantification were based on response factors relative to standards subjected to the same hydrolytic procedure (Bellinger et al. 2005). Monosaccharide distributions are expressed as the relative abundance (RA) of individual monosaccharides within the total analyzed fraction.

Quantification of carbohydrate hydrolyzing activity by extracellular enzymes—Hydrolysis by extracellular enzymes of sediment carbohydrates to generate newly produced reducing sugars was quantified by use of the method of Miller (1959). Triplicate surface 2-mm sediment samples were collected from each time point, frozen on site, and thawed for analysis. Increases in reducing sugar concentration within the sediment pore water were measured over a time course. At the beginning of each time course, the metabolic inhibitor sodium azide was added to the slurry to prevent new microbial action. The increase of reducing sugars then depends on the concentration of extracellular hydrolyzing enzymes present at the time the sample was taken, rather than subsequent enhanced microbial action within the slurry. One gram (wet weight) of thawed sediment, 13.5 mL of dH_2O, and 0.5 mL of sodium azide (0.2% final concentation NaN_3) were placed in flasks ($n = 3$) and subjected to a time course incubation (25°C, 140 rotations per minute) with samples extracted at 0 h, 1 h, 2 h, 3 h, and 4 h. At each time interval, 1 mL of supernatant was extracted, centrifuged (3,000 × g for 3 min), and diluted (1:10). Two hundred microliters of reagent A (16 g anhydrous sodium carbonate, 0.9 g potassium cyanide made up to 1 L with dH_2O) and reagent B (0.5 g L^{-1} potassium ferric hexacyanide) were added to 200 μL of diluted sample, mixed, and boiled (100°C) for 15 min. Samples were cooled in a water bath (20°C) for 5 min, and 1 mL of reagent C (1.5 g ferric ammonium sulphate, 1 g sodium dodecyl sulphate, 4.2 mL of concentrated H_2SO_4 made up to 1 L with dH_2O) was added to the samples, which were left to stand for 1 h. Samples were measured spectrophotometrically at 690 nm (Miller 1959). Sugar release by extracellular enzymes was quantified as glucose equivalents.

Total nucleic acid extraction from sediments—Total nucleic acids (DNA and RNA) were extracted from 0.1-g aliquots of sediment essentially as described by Osborn et al. (2000) with the nucleic acid pellets resuspended in 100 μL of diethylprocarbonate (DEPC)-treated water. Total nucleic acids were visualized by agarose gel electrophoresis, in 0.7% (w/v) agarose triphosphate EDTA gels.

RT-PCR amplification of 16S rRNA—Total RNA was prepared by digestion of 30 μL of total nucleic acids by use of DNase in the presence of DNase buffer containing 1 mol L^{-1} sodium acetate and 50 mmol L^{-1} $MgSO_4$ (pH 5.0), in accordance with manufacturer's instructions (Roche). cDNA of 16S rRNA was generated by use of primer 518R (5′-CGTATTACCGCGGCTGCTCG-3′) with Superscript III according to manufacturer's instructions (Invitrogen) before PCR amplification using the labelled primers FAM63F (5′-CAGGCCTAACACATGCAAGTC-3′) and HEX518R (see above) (Girvan et al. 2003). Reaction mixtures contained 200 mmol L^{-1} of each deoxyribonucleotide triphosphate (dATP, dCTP, dGTP, and dTTP) (Roche), 5 μL of 10× PCR buffer (KCl, $[NH_4]_2SO_4$, Qiagen) 5 μL of α-casein (1 mg mL^{-1}), 2.5 U of *Taq* DNA polymerase (Qiagen), 20 pmol of each primer, 1 μL of template DNA, and DEPC-treated water to a final volume of 50 μL. PCR amplification was carried out by use of an initial denaturation at 95°C for 5 min; 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; and a final extension step of 72°C for 10 min. PCR products were analyzed by electrophoresis of 5 μL of PCR product in a 1% (w/v) agarose gel.

Terminal restriction length polymorphism analysis—Amplified 16S rRNA genes were purified with a QIAquick PCR purification kit (Qiagen) and digested separately with 10 U of *Alu*I and *Cfo*I (Roche) at 37°C for 3 h. Restriction digests were mixed with 12 μL of deionized formamide and 0.5 μL of ROX-labelled Genescan 500-bp internal size standard (Applied Biosystems) and denatured by boiling for 5 min and then immediately transferred to ice. Duplicate samples were electrophoresed on an ABI 310 genetic analyzer (Applied Biosystems) at 15.0 V for 30 min, with resulting profiles analyzed by use of Genescan v3.1 software (Applied Biosystems). Duplicate profiles were then compared by use of T-Align (Smith et al. unpubl. data). The RA of each individual terminal restriction fragment (T-RF) was calculated from its peak area by use of the following equation: relative

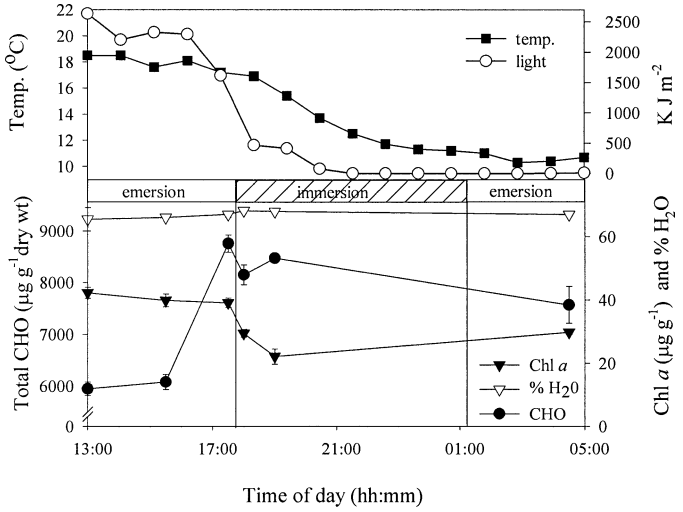

Fig. 1. Temperature (°C), irradiance (KJ m^{-2}), total carbohydrate (CHO; μg g^{-1}), chlorophyll *a* (μg g^{-1}), and sediment water content (%) in the top 2 mm of sediment at Alresford Creek, Colne Estuary, United Kingdom, measured over a 16-h period over a tidal emersion–immersion–emersion cycle on 27–28 May 2003. Values are mean ± SE, $n = 20$.

peak area for each T-RF = peak area of each T-RF/total peak area of all T-RFs in the terminal restriction length polymorphism (T-RFLP) profile.

Statistical analysis—Temporal changes in sediment carbohydrate and Chl *a* data were analyzed by use of ANOVA. Data were checked for normality by Bartlett's test and where necessary were transformed before analysis. There were no significant differences between quadrats sampled at the same time point, and mean values (±standard errors, $n = 20$) are presented. Significant differences between sample times were determined when $F_{5,119} > 2.29$ for the whole data set, or $F_{2,59} > 3.159$ when changes either over the emersion period or between the end of the emersion and the first 1.5 h of immersion were considered (individual *F* values are not presented for brevity). Where significant differences were found, post-hoc Tukey tests were used to determine differences between individual time points. Multivariate analysis of RA of monosaccharide profiles and relative peak area as a measure of abundance for individual T-RFs within the active bacterial community composition were carried out by use of Multivariate Statistical Package v3.13b (Kovcomp. com). All RA data were transformed (log ratio for Principal Component Analysis [PCA], arcsin for ANOVA) to remove the effects of closure (MVSP) (Zar 1999). Temporal changes in presence, absence, or RA of individual T-RFs were determined after nesting quadrat data within temporal data (Zar 1999). Statistical analyses were conducted by use of MINITAB ver. 13.3.

Results

Environmental conditions May 2003—Light levels remained relatively constant between 13:00 and 16:00 h, before declining. It was dark 3 h after the site had been immersed, and sampling in the following emersion period was conducted in darkness. Daytime temperature was ~18°C, decreasing to 11°C during the night (Fig. 1). Chl *a* content in the top 2 mm of sediment ranged from 22–42 μg g^{-1} over the 15.5-h sampling period (Fig. 1). Chl *a* content did not significantly vary over the low-tide period but declined significantly 30 min and 1.5 h after tidal immersion (a 42% loss, $p < 0.001$). Chl *a* content did not vary significantly from early high tide to the following low tide (Fig. 1). Sediment water content in the top 2 mm varied between 65% and 69%, and there were no significant differences over the 15.5-h sampling period (Fig. 1). No nutrient limitation was apparent over the low-tide or high-tide periods, with nutrient ratios remaining relatively stable. Pore water nitrate, phosphate, and silicate concentrations were from 156–202 μmol L^{-1}, 7–11 μmol L^{-1}, and 39–57 μmol L^{-1}, respectively, and there were no significant temporal changes in N : P, N : Si or P : Si ratios (Table 1).

Tidal changes in water soluble and bound carbohydrate fractions—Total carbohydrate content (μg g^{-1}) increased significantly by 47% during low tide, reaching a maximum concentration of 8754 μg g^{-1} at the end of the low-tide period ($p < 0.001$; Fig 1). After tidal cover, total carbohydrate content remained relatively stable. There were no significant correlations between Chl *a* and total carbohydrate content during low-tide periods.

There were significant temporal changes in water-soluble carbohydrate fractions over the tidal cycle. Low-tide colloidal carbohydrate content (μg g^{-1} and μg Chl a^{-1}) increased significantly ($p < 0.01$), reaching a maximum content of 682 μg g^{-1} by the end of low tide (Fig. 2A). The rate of colloidal carbohydrate increase over the emersion period was 2.03 ± 0.59 μg glucose equivalents μg Chl a^{-1} h^{-1} (Fig. 2B). After

Table 1. Pore water (top 2 mm sediment), nutrient content (μmol L^{-1}), and stoichiometric ratios (N : P, N : Si, and P : Si) measured during a tidal emersion-immersion-emersion cycle at Alresford Creek, Colne Estuary, United Kingdom, on 27–28 May 2003.

Time	$NO_3^- + NO_2^-$ (μmol L^{-1})	N : P ratio	Phosphate (μmol L^{-1})	N : Si ratio	Silicate (μmol L^{-1})	P : Si ratio
13 : 00 h	202.49±19.4	25.34± 3.48	7.99±0.9	5.13±0.62	39.51±4.0	0.20±0.03
15 : 30 h	197.19±17.7	20.78±1.79	9.49±0.7	4.90±0.51	40.22±3.3	0.23±0.02
17 : 30 h	193.39±17.0	23.67±2.15	8.17±0.8	4.29±1.70	45.04±4.9	0.18±0.03
18 : 00 h	196.59±11.4	17.73±1.78	11.09±1.6	3.76±0.58	52.31±3.9	0.21±0.02
19 : 00 h	160.70±16.3	17.88±1.77	8.99±0.5	2.81±0.31	57.21±3.2	0.16±0.02
04 : 30 h	156.78±14.1	20.49±3.89	7.65±0.8	3.43±0.48	45.67±5.0	0.17±0.03

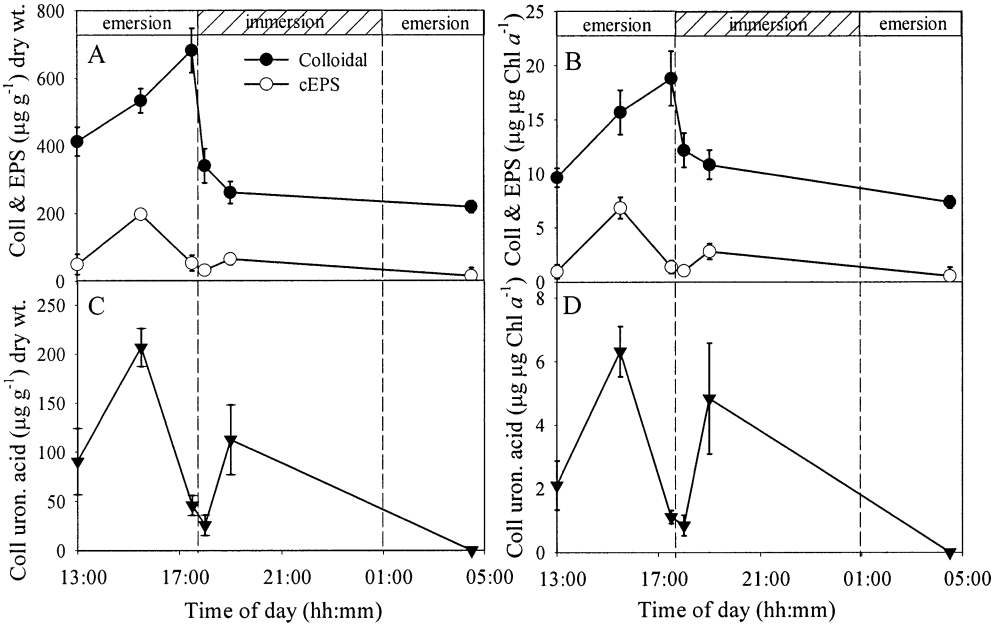

Fig. 2. Changes in colloidal carbohydrate and cEPS content in (A) μg glucose equivalents g^{-1} dry weight of sediment, (B) normalized to chlorophyll *a*, (C) colloidal uronic acid fraction in μg g^{-1}, and (D) normalized to chlorophyll *a* measured over a 16-h period over a tidal emersion–immersion–emersion cycle on 27–28 May 2003. Values are mean ± SE, $n = 20$.

30 min of tidal cover, colloidal content decreased significantly ($p < 0.001$), and within 1.5 h of tidal cover, 62% of colloidal carbohydrate was lost compared with the content at the point of tidal immersion. After this initial loss, colloidal concentrations had not changed significantly by the time of the next sampling, during a night-time tidal emersion period (Fig. 2A,B).

The amount of cEPS increased significantly during low tide ($p < 0.001$), reaching a maximum content of 198 μg g^{-1}; an increase of 300% within a 2.5-h exposure period (Fig. 2A). After the peak in cEPS during mid afternoon, concentrations decreased, and there was not a significant decrease even after 30-min tidal cover. However, after a further hour of tidal cover, cEPS concentrations increased significantly ($p < 0.05$). cEPS was barely detectable (15 μg g^{-1}) after exposure at the next tidal phase (Fig. 2A,B).

The uronic acid content of the colloidal fraction showed a similar pattern of change to the cEPS subfraction of colloidal ($r = 0.94$, $p < 0.001$, $n = 6$) rather than to the overall colloidal carbohydrate pool. After 2.5 h of exposure, colloidal uronic content increased significantly ($p < 0.001$), reaching a maximum content of 207 μg g^{-1}, an increase of 128% (Fig. 2C). After decreases during the latter period of tidal exposure and the first 30 min of tidal cover, colloidal uronic content increased significantly after 1.5 h of tidal cover ($p < 0.001$), a 348% increase from early phase tidal cover (30 min). Uronic acid was not present within the colloidal carbohydrate fraction after exposure at the next tidal phase (Fig. 2C,D). Biomass-normalized colloidal uronic content showed the same overall trends (Fig. 2D).

HW carbohydrate content showed a decrease followed by an increase over low-tide exposure, with a significant biomass-normalized rate of production (1.92 ± 0.9 μg glucose equivalents μg Chl a^{-1} h^{-1}) over the emersion period (Fig. 3A,B). After 30 min of tidal cover, HW carbohydrate content decreased significantly by 33% ($p < 0.01$). Following a further 1-h of tidal cover, the amount of HW-extracted carbohydrates increased significantly ($p < 0.01$) by 26%. HW carbohydrate content at the beginning of the next tidal emersion period was not significantly different from the measurement made after 90 min of tidal immersion. During the first tidal emersion period, the uronic acid concentrations in the HW fractions increased steadily, reaching a maximum of 509 μg g^{-1}; an increase of 66% from the beginning of the emersion period ($p < 0.001$; Fig. 3C). Increases in HW uronic acid content matched those of the HW carbohydrate fraction. HW uronic content showed a strong correlation with colloidal carbohydrates fractions ($r = 0.96$, $p < 0.001$, $n = 6$). High-tide HW uronic content also increased after 1.5 h immersion ($p < 0.001$; Fig. 3C,D).

There was no significant variation in overall HB carbohydrate content over the tidal cycle period. However, the uronic acid content of the HB fraction increased significantly (208%) at mid low tide ($p < 0.001$), reaching maximum concentrations of 398 μg g^{-1} (Fig. 3C), and significantly correlating with cEPS content ($r = 0.97$, $p < 0.001$, $n = 6$). HB uronic content increased after 30 min of tidal cover ($p < 0.001$), to a maximum of 407 μg g^{-1}. One hour of further tidal cover led to a significant decrease in HB uronic content ($p < 0.001$) to preimmersion values.

Changes in sediment carbohydrates through an emersion-immersion-cycle during June—Light levels decreased during the daytime emersion period in June, and were on average lower than in May, although temperature was higher (Fig. 4). Chl *a* content (data not shown) showed a 14.2% increase, from 37.3 ± 2.9 to 42.6 ± 2.2 μg g^{-1} during the emersion period. There was no significant loss of Chl *a* immediately

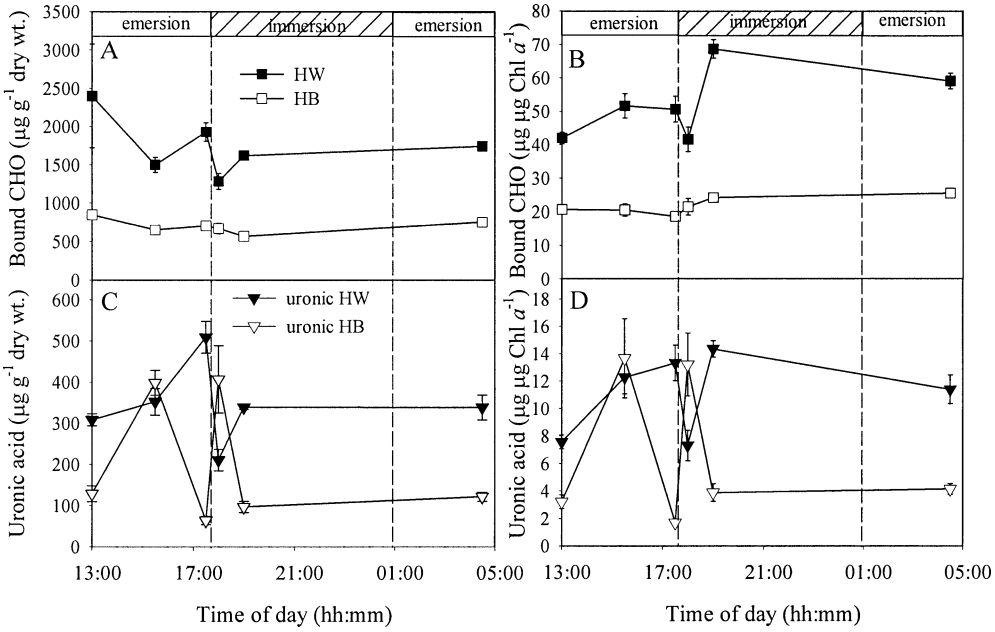

Fig. 3. Changes in (A) Hot water (HW)-extracted and hot bicarbonate (HB)-extracted carbohydrate content (μg g^{-1}) and (B) normalized to chlorophyll *a*, (C) HW- and HB-extracted uronic acid content (μg g^{-1}), and (D) normalized to chlorophyll *a* measured over a 16-h period over a tidal emersion–immersion–emersion cycle on 27–28 May 2003. Values are mean ± SE, n = 20. Uronic HW, HW-extracted uronic acids; uronic HB, HB-extracted uronic acids.

after immersion, but after 90 min of tidal cover, Chl *a* content had dropped by 21.6% to 35.6 ± 2.1 μg g^{-1}. Total carbohydrate content increased over tidal emersion, but there were no significant increases in colloidal or cEPS during this time (Fig. 4). After 90 min of tidal cover, there was a 5.7% loss of colloidal and a 25% loss of cEPS. HW carbohydrates rose significantly over the tidal emersion period, whereas HB carbohydrate content fell. Both these fractions increased after tidal immersion, by 15% and 25%, respectively (Fig. 4).

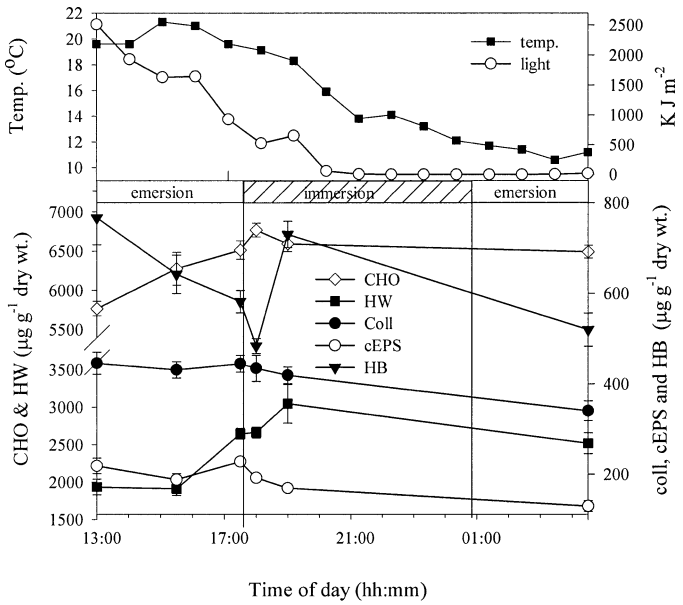

Fig. 4. Temperature (°C), irradiance (KJ m^{-2}), and total carbohydrate (CHO), colloidal (Coll), cEPS, and hot water (HW)-extracted and hot bicarbonate (HB)-extracted carbohydrate contents (μg g^{-1}) in the top 2 mm of sediment measured over a 16-h period during a tidal emersion–immersion–emersion cycle on an intertidal mudflat on 11–12 June 2003. Values are mean ± SE, n = 20.

Tidal changes in monosaccharide components and distribution—RA of monosaccharides within each extracted carbohydrate fraction fluctuated over the tidal cycle with glucose being the most abundant monosaccharide component of the cEPS and HW-extracted carbohydrate fractions (Fig. 5). RA of glucose in the cEPS fraction was between 40% and 60% in May but was lower in June, between 20% and 40% RA (Fig. 5D). The RA of glucose in cEPS increased mid low tide (Fig. 5A) in May, thereafter decreasing toward the end of low-tide exposure. After 1.5 h of tidal immersion, glucose RA increased, with a return to early low-tide RA values at the next tidal phase. In the June samples, the RA of glucose in cEPS was higher during tidal exposure compared with during tidal immersion (Fig. 5D). The other major sugars in cEPS were galactose, xylose, fucose, and mannose. A low RA of ribose (2–4%) was present in two of the May cEPS samples, but otherwise, ribose was not present in the May or June cEPS samples. The HW carbohydrate fractions had higher RA of glucose, between 50% and 75% (average, 66%) of the total monosaccharide composition over the tidal cycle in May (Fig. 5B). RA of glucose in the HW fraction increased over the low-tide period, peaking at late low tide in both May and June (76% and 66%, respectively; Fig. 5B,E). Glucose RA in the HW fraction decreased with the onset of high tide. The HB fraction showed the most evenly distributed proportions of other sugars of all three fractions, with glucose averaging 28% and galactose, mannose, fucose, and rhamnose averaging (combined) 46% of

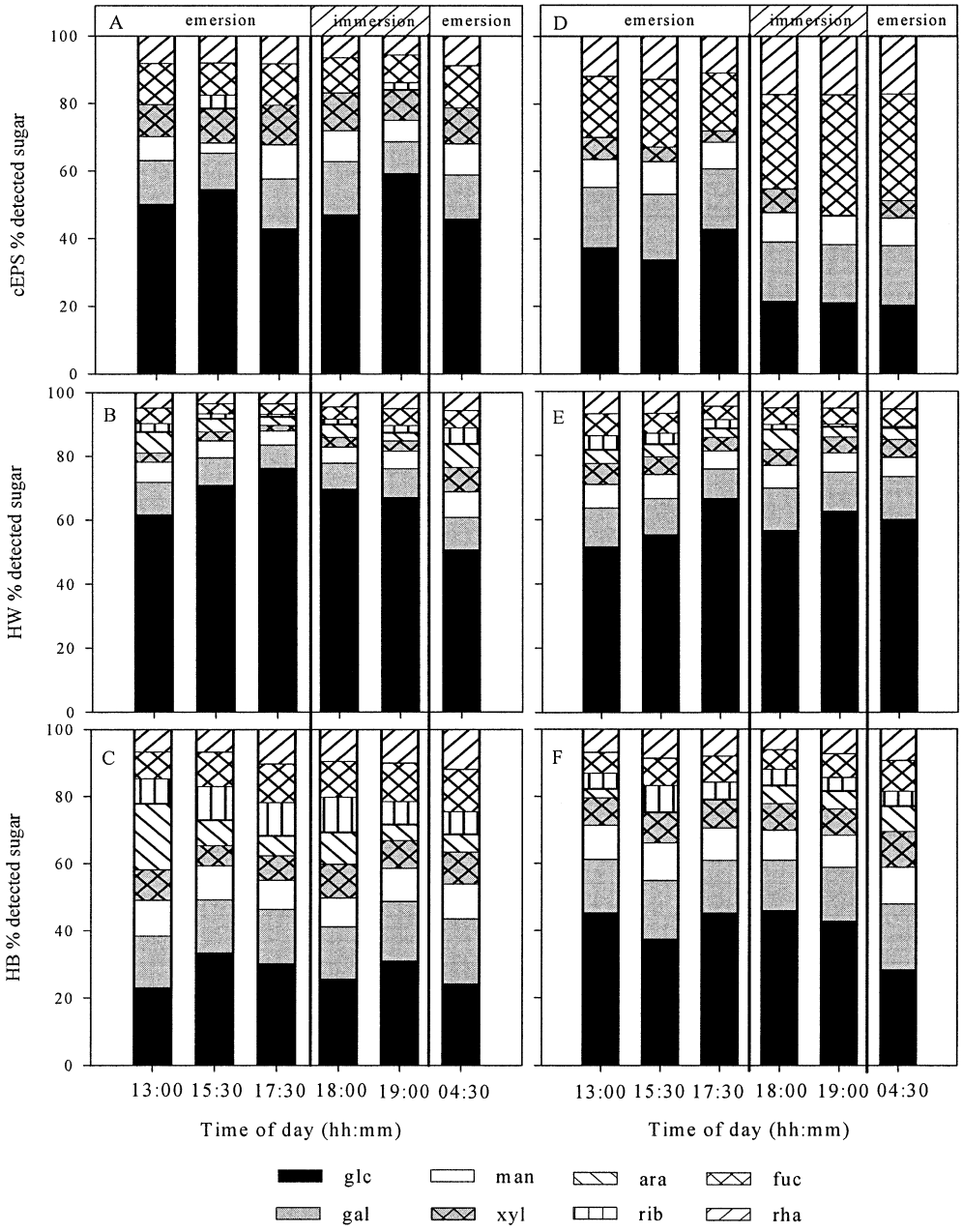

Fig. 5. Monosaccharide composition (% of total fraction) of (A, D) cEPS, (B, E) hot water–extracted, and (C, F) hot bicarbonate–extracted carbohydrates extracted from intertidal sediments of the Colne Estuary, United Kingdom, sampled at early, mid, and late low tide, early phase of tidal cover (30 min and 1.5 h) and next tidal phase on 27–28 May (A–C) and 11–12 June (E–F) 2003. glc, glucose; gal, galactose; man, mannose; xyl, xylose; ara, arabinose; rib, ribose; fuc, fucose; rha, rhamnose. Values are % means, $n = 6$). Monosaccharides were measured by gas chromatography/mass spectroscopy analysis.

the monosaccharide composition over the tidal cycle in May (Fig. 5C). In the June samples, the average RA of glucose was higher (41%). The HB fraction contained between 4% and 10% ribose, and ribose was consistently found in all HB samples (Fig. 5C,F).

Principal components 1 and 2 from PCA of the May and June monosaccharide data explained 70.7% of the variability in the data (Fig. 6). The three carbohydrate fractions were generally separated on the plot on the basis of their dominant monosaccharides. Sugar components of the cEPS fraction were more diverse, with a broad scatter along the PC1 axis of glucose and galactose. Daylight emersion samples of cEPS in May (13:00 h M1 and 15:30 h M2 samples) showed enrichment with glucose (as did the 19:00 h M5 sample). Compared with the May samples, June cEPS showed greater enrichment with rhamnose and fucose, especially samples J4–J6 taken after the daytime emersion period (Fig. 6). Scores showed a trend toward predominately glucose-rich HW carbohydrates (Fig. 6). There was a very clear increase in glucose enrichment in the HW fraction during the expo-

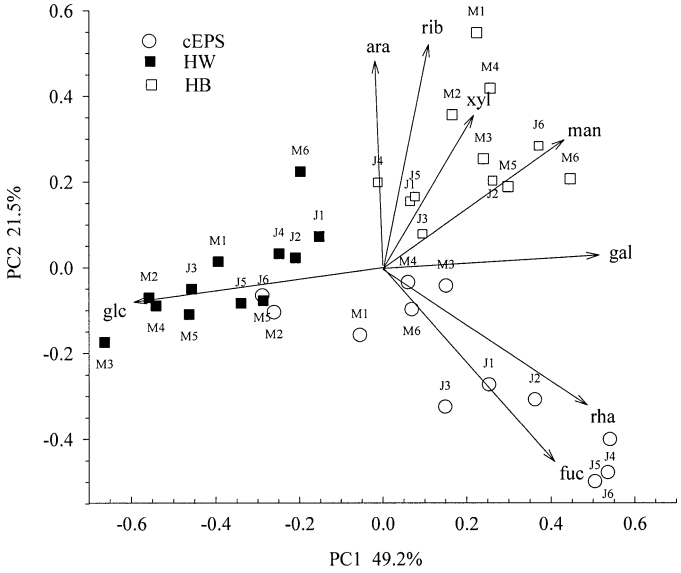

Fig. 6. Scatter plot of sample scores on principle components 1 and 2 from a PCA of the monosaccharide composition (relative abundance) of cEPS, HW, and HB fractions of intertidal sediment carbohydrates samples during two different 16-h emersion–immersion–emersion periods on 27–28 May (M) and 11–12 June (J) 2003 at Alresford Creek and Wivenhoe on the Colne Estuary, United Kingdom. (Vectors: glc, glucose; gal, galactose; man, mannose; xyl, xylose; ara, arabinose; rib, ribose; fuc, fucose; rha, rhamnose). Numbers relate to sampling time points: 1, 13:00 h; 2, 15:30 h; 3, 17:30 h; 4, 18:00 h; 5, 19:30 h; and 6, 04:30 h.

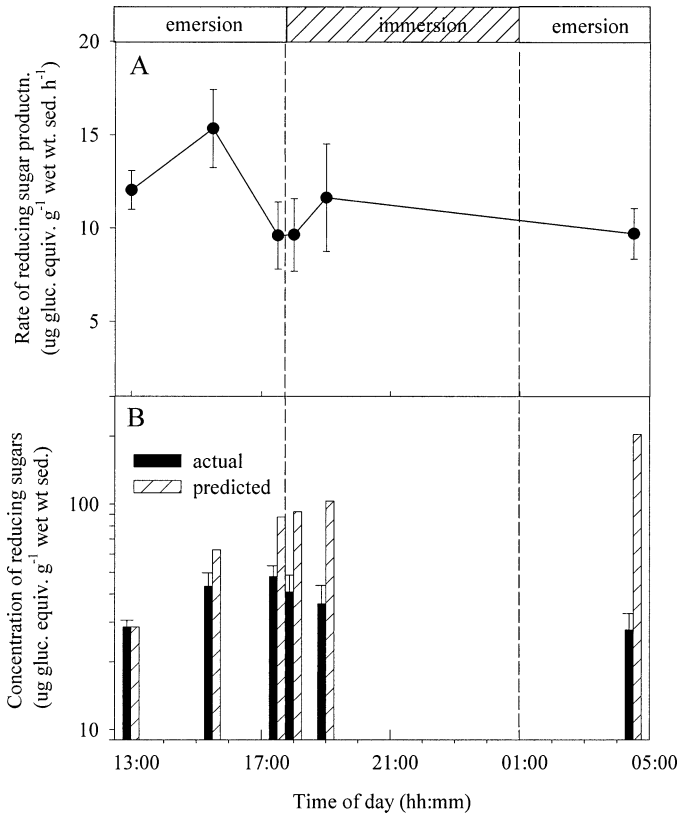

Fig. 7. (A) Rate of production of reducing sugars (μg glucose equivalents g^{-1} wet weight of sediment h^{-1}) in intertidal sediments by extracellular enzymatic activity over a tidal emersion-immersion-emersion cycle on 27–28 May 2003 at Alresford Creek, Colne Estuary, United Kingdom, calculated from changes in reducing sugar concentration in abiotic sediment slurries over a 4-h incubation (mean ± SE, $n = 12$). (B) Concentration of reducing sugars in intertidal sediment (mean ± SE, $n = 3$) and predicted sediment concentration applying the rates of production given in Fig. 7A and assuming no loss.

sure period (M1–M3, J1–J3) in both May and June, followed by a decrease during tidal cover and darkness. HB carbohydrates were enriched with ribose, xylose, and mannose, with an increasing mannose component as the tidal cycle progressed.

Changes in extracellular hydrolysing enzyme activity and reducing sugar concentrations—The rate of production of reducing sugars (μg glucose equivalents g^{-1} wet weight of sediment h^{-1}) by extracellular hydrolytic activity increased to its highest rate at mid low tide (15:30 h), before declining (Fig. 7A). By the time of the final sample point (tidal emersion, 04:30 h), production rates had decreased to levels below those of the initial rate at the beginning of the illuminated emersion period (13:00 h). The rate of reducing sugar production was significantly correlated with sediment cEPS, colloidal uronic acid, and HB-extracted uronic acid content ($r = 0.92$, 0.96, and 0.97, respectively; all $p < 0.001$; $n = 6$). The in situ pore water concentrations of reducing sugars in the thawed sediments (the initial concentration of each time-course experiment) also increased over the illuminated emersion period, before declining during tidal immersion and darkness (Fig. 7B). Sediment reducing sugar content was significantly correlated with colloidal carbohydrate ($r = 0.75$, $p < 0.05$, $n = 6$). Predicted pore water–reducing sugar concentrations were calculated from the average rate of sugar release between two sample points (from Fig. 7A) and the time intervals between sample points. The predicted concentrations would be what would be expected if no loss occurred of the newly produced reducing sugars. There is a substantial mismatch between the predicted and actual concentrations of reducing sugars over the emersion–immersion–emersion cycle. This indicates high potential loss rates of this carbohydrate component, because of microbial activity and/or wash away during tidal cover.

Bacterial community dynamics during the diurnal cycle—T-RFLP profiles were generated from the active bacterial community (rRNA) at six time points during the tidal cycle. A total of 59 T-RFs were identified, with an average of 33.9 ± 0.89 (standard error) T-RFs per sample. There was no significant difference in community richness between samples. Principal components 1 and 2 derived from PCA of the active community data identified by T-RFLP explained 36.9% of the variability in the data. The active bacterial communities did not generally form discrete clusters with respect to the six sample times (except for samples taken at 17:30 h and 04:30 h; Fig. 8). Replicate T-RFLP profiles derived from RT-PCR products from individual RNA samples within quadrats did, however, cluster together. There was no

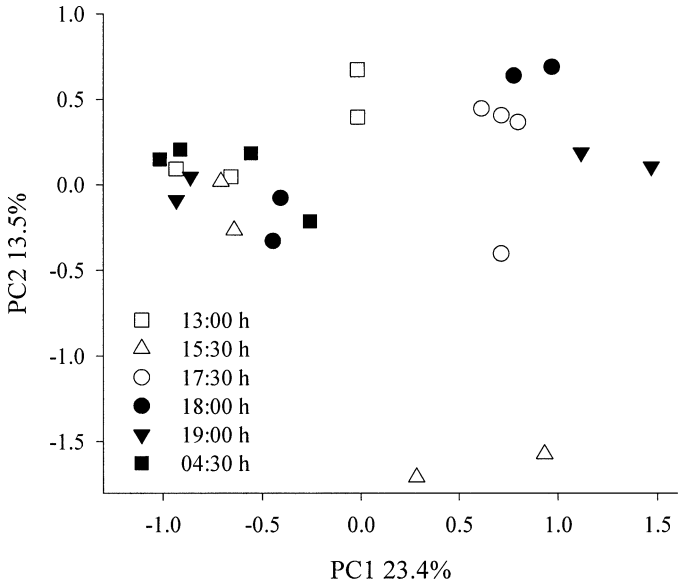

Fig. 8. Principal components ordering of data (axes 1 and 2) generated from the replicate 16S rRNA T-RFLP profiles describing the active bacterial communities present in the top 2 mm of sediment from Alresford Creek during 27–28 May 2003.

clear pattern of any consistent temporal shift or clustering with respect to the time of sampling during the tidal cycle. In addition, there were no significant correlations found between the overall community structure of the active bacterial community data (PC axes scores of samples) and the concentrations of carbohydrates, uronic acids, algal biomass, or rates of extracellular enzyme activity (data not shown).

Analysis of RA data for individual T-RFs found that 12 T-RFs out of the total 59 did exhibit significant changes in RA with time. Five different response patterns were identified, with between one and five R-TFs falling into each category (Table 2). The RA of three T-RFs (113.09, 117.36, and 245.05) that were present throughout the time course were significantly correlated with the rate of hydrolysis of sediment carbohydrates by extracellular enzymes to generate newly produced reducing sugars (Table 2). Two other T-RFs (170.56, 216.84) were only present at 15:30 h, when the rate of enzymatic activity was highest.

Discussion

Changes in sediment carbohydrate fractions over time—Colloidal extracts from estuarine sediments contain a broad size range of carbohydrate materials, from mono- and oligosaccharides through to polymers >100 kD in size (Stal 2003; Underwood and Paterson 2003). Precipitation of EPS from such material, using 70% v/v ethanol, has been widely used (Decho 1990; Smith and Underwood 1998; Underwood and Paterson 2003), although with some uncertainty as to which components of the colloidal material are actually precipitated (Stal 2003). A number of compositionally different EPS can be extracted from cEPS extracts (de Brouwer and Stal 2001; Magaletti et al. 2004; Underwood et al. 2004), emphasizing the operational nature of these extraction procedures (Underwood and Paterson 2003). Despite these uncertainties, clear patterns in changes in the concentrations of water-soluble carbohydrate fractions on intertidal mudflats over tidal emersion periods, related to microphytobenthic activity are well described. There is usually a close relationship between the rate of photosynthesis and increases in colloidal carbohydrates, particularly the smaller-molecular-weight carbohydrate fractions (Staats et al. 2000; de Brouwer and Stal 2001), resulting in linear increases in colloidal and cEPS

Table 2. Terminal restriction fragments (T-RFs) derived from T-RFLP rRNA profiles of intertidal sediment (top 2 mm) from Alresford Creek, Colne Estuary, United Kingdom, showing significant patterns of change in relative abundance (RA) of T-RF peak area over the course of a tidal emersion-immersion-emersion period from 27–28 May 2003. Forty-six other T-RFs were identified in the profiles, but these showed no signficant changes in RA over the period of study. T-RF fragment length, summary of pattern, significance of change, and of correlation with enzyme activity (correl. enzyme activity) are shown.

Pattern of RA change	T-RF size (nucleotides)	Change in RA, signficance level
Decline over period	39.06	decline only over first emersion period, $F_{2,9}=7.1$, $p<0.05$
	159.24	from 3.9% to 1.3% RA, then increase to 5.6%, $F_{5,6,12}=186.1$, $p<0.001$
	214.72	variable, from 20% down to 5%, then increase to 14–18%, $F_{5,6,12}=25.2$, $p<0.001$
Increase over period	194.29	increase, from 3.1 up to 7.5–8.1% during immersion, $F_{5,6,12}=11.9$, $p<0.001$
Highest RA at 15:30 h (mid low tide)	113.09	from 1–2% to 4.1% RA, $F_{5,6,12}=6.85$, $p<0.01$; correl. enzyme activity, $r=0.73$, $p<0.05$
	117.36	from 1.3% to 3.4% RA, $F_{5,6,12}=14.1$, $p<0.001$; correl. enzyme activity, $r=0.85$, $p<0.01$
	170.56	only present at 15:30 h, RA=0.5%, $F_{5,6,12}=3.43$, $p<0.05$
	216.84	only present at 15:30 h, RA=1.5%, $F_{5,6,12}=6.13$, $p<0.01$
	245.05	from 3.9% to 6.8% RA, $F_{5,6,12}=66.5$, $p<0.001$; correl. enzyme activity, $r=0.83$, $p<0.05$
Highest RA at 17:30 h (just before immersion)	225	1% RA at 17:30 h , otherwise < 0.1%, $F_{5,6,12}=6.13$, $p<0.01$
	243.1	1% RA at 17:30 h, otherwise < 0.05–0.01%, $F_{5,6,12}=9.86$, $p<0.001$
Highest RA at 04:30 h (second emersion period)	155.69	7.8% RA at 04:30 h, rest <0.3%, $F_{5,6,12}=2346$, $p<0.001$

content over tidal emersion periods (Stal 2003; Underwood and Paterson 2003). cEPS dynamics are more complex, with production rates and carbon allocation patterns showing varying patterns, but generally in a thick biofilm, the amounts of colloidal and cEPS would be expected to increase over a tidal exposure period. This is the pattern seen clearly in the colloidal and, to some extent, in the cEPS data over the tidal emersion period in May (Fig. 2). The rates of increase in colloidal and cEPS ($\sim$2 μg glucose equivalents μg Chl a^{-1} h^{-1}) fall into the range reported in other field and culture studies (Underwood and Paterson 2003). Nutrient limitation can also result in significantly increased carbohydrate production (overflow hypothesis) (Stal 2003; Underwood and Paterson 2003). However, the pore water concentrations of nutrients and the stoichiometric ratios calculated here suggest nutrient limitation was unlikely to be influencing EPS production within the biofilms.

During June, colloidal and cEPS content remained relatively constant over the immersion period rather than showing expected increases. Although Chl *a* content was similar, light levels decreased more rapidly during the exposure period in June compared with May. This would have reduced the overall rate of colloidal carbohydrate production (Smith and Underwood 1998). However, increases in total and HW fractions and in the RA of glucose in the HW fraction (Fig. 6) indicate that primary production was occurring (de Brouwer and Stal 2001; Underwood et al. 2004). However, loss rates of colloidal exudates within sediments can be high (see following), and if production rates of colloidal and cEPS matched losses within the sediment, then no net change would be seen. This mismatch between the flux of carbon through particular pools and corresponding concentration data patterns was shown in ^{14}C-labelling studies on the Humber Estuary, where concentrations of EPS and colloidal carbohydrates decreased over an afternoon tidal exposure period, despite active production of colloidal and cEPS (Smith and Underwood 1998; Underwood and Smith 1998*b*). Recent data have also shown that biofilm activity is not necessarily linearly dependent on light but is affected by the temporal patterns of species replacement, surface micromigration, and endogenous rhythms of photoacclimation over tidal emersion periods (Underwood et al. 2005). Such processes can result in no net change in certain variables, despite active fluxes of material through these pools (Underwood 2002). Perkins et al. (2003) have also recently shown that sediment dewatering on intertidal flats can have a significant effect on the values of measured sediment variables, decreasing Chl *a* content (μg g^{-1}) by between 12% and 14% and increasing concentration (μg cm^{-2}) data by up to 40%, whereas colloidal carbohydrate data showed opposite trends. The physical effects of dewatering on resultant content and concentration data are greater the thinner the depth slice of sediment taken and potentially can either mask or emphasise real changes occurring in the sediments. Although we found no significant change in water content in our 2-mm-deep sediment slices over the course of the measurements, any dewatering, if significant, would serve to reduce the magnitude of any changes observed. Thus, the June data, although not showing such clear patterns of low tide–carbohydrate production as was found in May, are not incompatible with such an interpretation.

Sequential extraction of colloidal, HW-, and HB-soluble carbohydrate fractions resulted in $\sim$50% of the total sediment carbohydrate pool being characterized. It is important to emphasise that these extraction procedures do not isolate specific polymer types but, within the spectrum of different EPS present in sediments, broadly separate different carbohydrates based on solubility and binding characteristics (Underwood and Paterson 2003; Bellinger et al. 2005). That these separations do isolate different components of EPS is shown by the differences in temporal dynamics between fractions and the grouping of fractions along independent component axes in the PCA based on monosaccharide profiles. Fractions tended to clustered together, regardless of sample month, although some variations were observed within fractions between months, a pattern of grouping similar to that reported for different carbohydrate fractions isolated from various types of intertidal estuarine biofilms (Bellinger et al. 2005). The monosaccharide composition of cEPS was similar to that of HB with regards to proportions of galactose, xylose, fucose, and rhamnose, most notably in May. In June, cEPS had decreased concentrations of glucose and dramatically greater fucose and rhamnose contents. Higher glucose content in EPS has been linked to photosynthetic activity (de Brouwer and Stal 2001), whereas fucose and rhamnose are more refractory sugar components (Giroldo et al. 2003). The differences in glucose content in the EPS between May and June may reflect the greater net accumulation of newly produced EPS over the emersion period in May. Giroldo et al. (2003) reported relative increases in the proportion of deoxy sugars during the degradation of planktonic diatom EPS exudates, as glucose was preferentially used. This hypothesis would explain the increased rhamnose, fucose, and galactose and the decreased glucose contents in cEPS after tidal cover, particular in June, when ''wash away'' of cEPS was less than in May. Rhamnose enrichment in water-soluble carbohydrates relative to bound carbohydrates has also been found in estuaries studied by de Brouwer et al. (2003). In both May and June, cEPS tended to have a lower mannose content and was generally lacking in arabinose. This is consistent with previous culture and field experiments that have found colloidal polymers to include, at most, only low levels of and, in some cases, no arabinose (Taylor et al. 1999; Bellinger et al. 2005). The cEPS fraction was enriched in glucose relative to HB soluble polymers, but temporal patterns of the gain or loss of glucose were identical in both cEPS and HB. The temporal changes in uronic acid content in colloidal extracts mirrored those of cEPS, strongly indicating a significant uronic acid component of cEPS. A high uronic acid content promotes cross-linking of polymer chains that can provide structural properties (Decho 2000; Stal 2003). Both cEPS and HB fractions showed substantial, and similar, temporal changes in uronic acid content, with a temporal shift of 30 min between peaks of uronic content in HB and cEPS after tidal immersion. These similarities lead us to hypothesize that a proportion of the cEPS fraction is derived from HB polymers produced by the diatoms.

All HW polymers were glucose dominated, however, glu-

cose comprised a smaller proportion of the polysaccharide in June when compared with May (50–65% vs. 60–75%, respectively). Although the presence of some other monosaccharides in the HW fraction indicates some incorporation of material from other carbohydrate pools, the patterns of change with illumination indicates that the HW fraction primarily represents solubilization of the diatom intracellular storage glucan chrysolaminarin (Chiovitti et al. 2003; Underwood et al. 2004). In both the May and June samplings, increases in glucose content (as a part of the total detected sugar total) were observed during daylight emersion, reflecting photosynthetic activity and storage (de Brouwer and Stal 2001). During immersion or the darkened-emersed period, the proportion of glucose decreased, probably owing to catabolism of glucan by the diatoms (Smith and Underwood 1998; Underwood et al. 2004). This close dependence on irradiance suggests that changes in HW content are a good proxy measure for diatom photosynthetic activity in sediments.

HB polymers showed dominance of xylose, mannose, and galactose (representing 30–40% of the total) and, to a lesser extent, rhamnose and fucose (10–20% of the overall total). In June, a greater proportion of the polysaccharide was glucose, and a reduced rhamnose and fucose content were observed. HB carbohydrates are closely associated with the diatom frustule, forming the primary mucilage coating around the silica cell wall (Chiovitti et al. 2003; Bellinger et al. 2005). The high proportions of mannose, xylose, and uronic acids indicate structural properties for the HB fraction (Wustman et al. 1997; Chiovitti et al. 2003). At the beginning of the exposure period, when irradiance levels were highest, HB carbohydrates tended to group along the mannose, xylose components. Goes et al. (1996) observed increases in these sugars and galactose within the structural carbohydrate pool for phytoplankton exposed to ultraviolet light. Increased amounts of glucose in structural polysaccharides were observed by Goes et al. (1996) in phytoplankton and in the bulk carbohydrate fraction as determined by Taylor et al. (1999). The composition of HB extracts from diatom cultures and sediments is remarkably similar (Bellinger et al. 2005; Abdullah et al. unpubl. data), given the potential for incorporation of non-diatom-derived polymers from other sediment components. There appears to be a pathway by which HB-extracted material becomes available in the cEPS pool (e.g., similarities in temporal changes in glucose and uronic acid content), either by solubilization from cell walls or by deposition as motility trails within the environment (Chiovitti et al. 2003; Higgins et al. 2003), followed by probable bacterial degradation. These processes require further investigation.

Removal and deposition of carbohydrates during tidal cover—The mean flow velocity of water across a mudflat is greatest at the leading edge of the rising tide, just immediately after a portion of mudflat is initially covered, after which mean flow velocity decreases. This causes a ''turbid tidal edge,'' with suspended solid concentrations as high as 6 g L^{-1} (Christie and Dyer 1998). This zone of high turbidity passes rapidly and within 90 s suspended solid load can fall to below 2 g L^{-1}. The turbid layer consists of organic debris, algal cells, biofilm layers, and bubbles. This is a mechanism by which materials are moved landward across a mudflat profile. Mean velocities and suspended solid loads decrease (down to 370 mg L^{-1}) as material settles out on the mudflats (Christie and Dyer 1998). Evidence for deposition of detrital material during early tidal cover was seen in both May and June, with increases in carbohydrate content in the second set of subtidal samples possibly owing to deposition of such material. This would explain increases in total, HW, and HB fractions in both the May and June data sets. Coagulation of DOM, particularly polysaccharides, can be induced through mixing and bubble scavenging, forming particles rich in the deoxysugars rhamnose and fucose, as well as covalently bound sulphate (Zhou et al. 1998, Giroldo et al. 2003). Such particles can then settle in the lower flow velocities present after the turbid tidal edge has passed. In June, cEPS sampled after tidal cover had dramatically greater fucose and rhamnose contents.

During the first 90 min of tidal cover, sediment Chl *a* content decreased by ~40% in May and 16% in June. MPB possesses endogenous rhythms of vertical migration, and migrates down into the sediments generally just before tidal cover (Consalvey et al. 2004). Migration occurs mainly within the top few hundred micrometers, and within 2-mm-depth slices, Chl *a* content does not significantly change with migration (de Brouwer and Stal 2001; Kelly et al. 2001). Therefore, decreases in Chl *a* content with tidal cover are unlikely to be owing to migration of cells below the sediment sampling depth but instead indicate loss to the water column. Up to 50% of MPB biomass have been estimated to be ''washed away'' during tidal cover (de Jonge and van Beusekom 1995), and physical removal of biofilm material can be seen occurring in the turbid tidal edge, as described by Christie and Dyer (1998). If we consider Chl *a* as a marker for biomass, then there is a greater loss of colloidal (62% loss in May and a 25% loss of cEPS in June) from the sediment than can be explained by physical removal. These decreases in colloidal carbohydrates are similar to recorded losses of 40–50% over complete tidal immersion periods (Underwood and Smith 1998; Orvain et al. 2003; Underwood and Paterson 2003), indicating that this major period of loss actually occurs soon after tidal cover. It is noticeable that greater loss of colloidal occurred in sediments with higher colloidal content. Colloidal and EPS material located on the top of the sediments in thicker biofilms during emersion (Underwood et al. 1995) may be more likely to be removed either physically (in parallel with cells/Chl *a*), by dissolution into the overlying water column, or be lost through bacterial utilization. Bacteria produce a range of extracellular enzymes (e.g., α- and β-glucosidases, aminopeptidases) to degrade extracellular polymers (Simon et al. 2002). However, cleavage of carbohydrates by glucosidases to monosaccharides is not always necessary, and bacteria can take up oligosaccharides by using glycoporins; this being more energetically efficient than is extracellular hydrolysis to monosaccharides (Meon and Jüttner 1999). Use of methylumbelliferyl-labelled substrates to determine enzyme activities (Romaní et al. 2004) measures the maximum potential release rate (owing to the use of saturating concentrations of labelled substrate), rather than direct mea-

surements of in situ rates (Arnosti 2000, Simon et al. 2002). Potential maximum β-glucosidase activity was found not to change on a Dutch mudflat over tidal emersion or immersion cycles, despite large changes in concentrations of low-molecular-weight carbohydrates and EPS (van Duyl et al. 1999). We used a different approach here, measuring the increase in reducing sugars as a product of hydrolysis of polymeric material present in the sediment rather than adding saturating concentrations of labelled substrate to determine potential maximum rates (Arnosti 2000). The reducing sugars detected may be monosaccharides but also end units of oligo- and polysaccharides exposed by hydrolysis of bonds along the polymer chain (Arnosti 2000). The rates of enzymatic hydrolysis varied over the course of the experiment, correlating with sediment cEPS, colloidal uronic acid, and HB-extracted uronic acid content. The rates of hydrolysis indicate substantial potential production of reducing sugars that was not reflected in the sediment standing stocks. The difference between in situ reducing sugar concentrations and production rates can be attributed to rapid bacterial use of these reducing sugar compounds. Low-molecular-weight saccharides are rapidly used in marine sediments (van Duyl et al. 1999, 2000; Middelburg et al. 2000; Köster et al. 2005), with bacterial production significantly correlated with low-molecular-weight compounds (van Duyl et al. 1999). Rapid bacterial uptake of low-molecular-weight material can explain the steep decreases in concentrations of low-molecular-weight MPB exudates in the absence of photosynthesis, particularly in sands (Underwood 2002). Loss of colloidal material during immersion accounted for almost 80–90% of the colloidal and EPS carbohydrate produced during the previous illuminated emersion phase; with ~50% of this washed away and the remainder either lost through dissolution or bacterial activity. These high turnover rates will result in only a slow net accumulation of material over time (Orvain et al. 2003).

There were no major changes in the active bacterial community composition in the biofilms over the 24-h period despite changes in the availability of diatom exudates. It is not clear the extent to which increased bacterial activity in organic sediments and flocs is owing to new gene expression in existing bacteria or selection of a new bacterial flora (DeLong et al. 1993). Muddy sediments are rich in organic matter, and the degree of algal-bacterial coupling has been shown to be less than that seen in sandy sediments with a lower organic matter content (Köster et al. 2005). Shifts in community composition with increased organic input have been recorded over longer time periods (Pinhassi et al. 1999; Logue et al. 2004), with the greatest community shifts in more oligotrophic environments (Eiler et al. 2003). Algal activity resulted in an approximate doubling of sediment carbohydrate content during the present study, but given that background carbohydrate levels were high, it seems unlikely that a 6–8 h period of microphytobenthic production of labile and less labile exudates would be sufficient to drive a large shift in active bacterial community composition (Eiler et al. 2003). However, 12 taxa, out of the total 59 T-RFs identified, did show significant temporal changes in activity over the experiment. Of these, five T-RFs correlated with the peak in sediment cEPS content, and three were significantly correlated with the rate of extracellular reducing-sugar release. Mesocosm enrichment studies of picobacterioplankton communities also showed that a few bacterial species had a greater response than the community as a whole (Pinhassi et al. 1999; Eiler et al. 2003). These results emphasize the importance of identifying taxa-specific responses rather than grouping all bacterial activity. The identity of these taxa has not been determined, but bacteria within the α-Proteobacteria, γ-Proteobacteria, Cytophaga, and Flavobacterium groups are likely candidates. These bacteria are important degraders and colonizers of aggregates and sediments and producers of hydrolytic extracellular enzymes (DeLong et al. 1993; Pinhassi et al. 1999). Although it is premature to suggest that the taxa identified in the present study were responsible for the extracellular hydrolytic activity, the correlations among particular T-RF abundances, enzyme rates, and cEPS and HB carbohydrate content are intriguing and suggest that specific algal–EPS–bacterial relationships may occur in intertidal biofilms. Identification and isolation of specific EPS-degrading bacteria is now potentially possible, and characterization of their extracellular enzymes will also provide information on the structure, hydrolysis points, and relationships between structurally complex EPS molecules.

The changes in carbohydrate content and composition over a tidal emersion–immersion period allows the development of a conceptual model for EPS dynamics in intertidal sediments. During illuminated tidal emersion periods, photosynthesis by diatom biofilms results in increasing concentrations of colloidal carbohydrates, cEPS, and intracellular carbohydrates (HW). Photosynthetic activity leads to a relative enrichment of glucose in the various sediment EPS fractions. The production of extracellular carbohydrates stimulates the activity of a subset of bacterial taxa within the sediments—activity correlating with extracellular hydrolytic activity and the dynamics of certain sediment carbohydrate fractions (colloidal, cEPS). This bacterial activity may be responsible for the conversion of tightly bound, complex EPS associated with diatom cells (HB fraction) into more labile cEPS present in sediments. Removal of biofilm biomass, and proportionally greater losses of colloidal carbohydrates, is a rapid process, occurring during the first 90 min of tidal cover, rather than gradually over the whole tidal immersion period. Losses of labile carbohydrates owing to tidal removal and bacterial action result in an increase in the relative importance of more tightly bound polymers within sediments, coupled with the deposition of detrital material (particles, flocs) onto the sediment surface later in the tidal phase. Overall daily rates of net EPS accumulation are therefore low, despite high rates of production and loss. The validity of this model of biofilm functioning needs to be tested under a wider range of environmental conditions, particularly where biofilms are stressed by extremes of salinity, irradiance, temperature, and nutrient concentrations.

References

ARNOSTI, C. 2000. Substrate specificity in polysaccharide hydrolysis: Contrasts between bottom water and sediments. Limnol. Oceanogr. **45:** 1112–1119.

——— AND D. J. REPETA. 1994. Oligosaccharide degradation by anaerobic marine bacteria: Characterization of an experimental

system to study polymer degradation in sediments. Limnol. Oceanogr. **39:** 1865–1877.

BELLINGER, B. J., A. S. ABDULLAHI, M. R. GRETZ, AND G. J. C. UNDERWOOD. 2005. Biofilm polymers: Relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms. Aquat. Microb. Ecol. **38:** 169–180.

CHIOVITTI, A., M. J. HIGGINS, R. E. HARPER, R. WETHERBEE, AND A. BACIC. 2003. The complex polysaccharides of the raphid diatom *Pinnularia viridis* (Bacillariophyceae). J. Phycol. **39:** 543–554.

CHIOVITTI, A., P. MOLINO, S. A. CRAWFORD, R. TENG, T. SPURCK, AND R. WETHERBEE. 2004. The glucans extracted with warm water from diatoms are mainly derived from intracellular chrysolaminaran and not extracellular polysaccharides. Eur. J. Phycol. **39:** 117–128.

CHRISTIE, M. C., AND K. R. DYER. 1998. Measurements of the turbid tidal edge over the Skeffling mudflats. Geological Society, London, Special Publications, **139:** 45–55.

CONSALVEY, M. C., D. M. PATERSON, AND G. J. C. UNDERWOOD. 2004. The ups and downs of life in a benthic biofilm: Migration of benthic diatoms. Diatom Res. **19:** 181–202.

DE BROUWER, J. F. C., E.M.G.T. DE DECKERE, AND L. J. STAL. 2003. Distribution of extracellular carbohydrates in three mudflats in west-Europe. Est. Coastal Shelf Sci. **56:** 313–324.

DE BROUWER, J. F. C., AND L. J. STAL. 2001. Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. Mar. Ecol. Progr. Ser. **218:** 33–44.

DECHO, A. W. 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr. Mar. Biol. Annu. Rev. **28:** 73–153.

———. 2000. Microbial biofilms in intertidal systems: An overview. Cont. Shelf Res. **20:** 1257–1274.

DE JONGE, V. N., AND J. E. E. VAN BEUSEKOM. 1995. Wind- and tide-induced resuspension of sediment microphytobenthos from the tidal flats in the Ems Estuary. Limnol. Oceanogr. **40:** 766–778.

DELONG, E. F., D. G. FRANKS, AND A. L. ALLDREDGE. 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. **38:** 924–934.

DONG, L. F., D. C. O. THORNTON, D. B. NEDWELL, AND G. J. C. UNDERWOOD. 2000. Denitrification in sediments of the River Colne Estuary, England. Mar. Ecol. Prog. Ser. **203:** 109–122.

DUBOIS, M., K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:** 350–356.

EILER, A., S. LANGENHEDER, S. BERTILSSON, AND L. J. TRANVIK. 2003. Heterotrophic bacterial growth efficiency and community structure and different natural organic carbon concentrations. Appl. Env. Microbiol. **69:** 3701–3709.

FELSKE, A., B. ENGELEN, U. NÜBEL, AND H. BACKHAUS. 1996. Direct ribosome isolation from soil to extract bacterial rRNA for community analysis. Appl. Environ. Microbiol. **62:** 4162–4167.

GIROLDO, D., A. A. H. VIERA, AND B. S. PAULSEN. 2003. Relative increase in deoxy sugars during microbial degradation of an extracellular polysaccharide release by a tropical freshwater *Thalassiosira* sp. (Bacillariophyceae). J. Phycol. **39:** 1109–1115.

GIRVAN M.S., J. BULLIMORE, J. N. PRETTY, A. M. OSBORN, AND A. S. BALL. 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl. Environ. Microbiol. **69:** 1800–1809.

GOES J. I., N. HANDA, S. TAGUCHI, T. HAMA, AND H. SAITO. 1996. Metabolism of neutral monosaccharide constituents of storage and structural carbohydrates in natural assemblages of marine phytoplankton exposed to ultraviolet radiation. Limnol. Oceanogr. **41:** 1478–1489.

HAMELS, I., H. MUSSCHE, K. SABBE, K. MUYLAERT, AND W. VYVERMAN. 2004. Evidence for constant and highly specific active food selection by benthic ciliates in mixed diatoms assemblages. Limnol. Oceanogr. **49:** 58–68.

HIGGINS, M. J., P. MOLINO, P. MULVANEY, AND R. WETHERBEE. 2003. The structure and nanomechanical properties of the adhesive mucilage that mediates diatom-substratum adhesion and motility. J. Phycol. **39:** 1181–1193.

HONEYWILL, C., D. M. PATERSON, AND S. E. HAGERTHEY. 2002. Determination of microphytobenthos biomass using pulse-amplitude modulated minimum fluorescence. Eur J. Phycol. **37:** 485–492.

KELLY, J. A., C. HONEYWILL, AND D. M. PATERSON. 2001. Microscale analysis of chlorophyll *a* in cohesive, intertidal sediments: The implications of microphytobenthos distribution. J. Mar. Biol. Assoc. U.K. **81:** 151–162.

KÖSTER, M., S. DAHLKE, AND L-A. MEYER-REIL. 2005. Microbial colonization and nutrient dynamics of sandy and muddy sediments in hypertrophic coastal waters of the southern Baltic Sea (Nordrügensche Bodden). Aquat. Microb. Ecol. **39:** 69–83.

LIU, W.T., T. L. MARSH, H. CHENG, AND L. J. FORNEY. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl. Environ. Microbiol. **63:** 4516–4522.

LOGUE, J. B., C. T. ROBINSON, C. MEIER, AND J. R. VAN DER MEER. 2004. Relationship between sediment organic matter, bacteria composition, and the ecosystem metabolism of alpine streams. Limnol. Oceanogr. **49:** 2001–2010.

LORENZEN, G. J. 1967. Determination of chlorophyll and phaeopigments: Spectrophotometric equations. Limnol. Oceanogr. **12:** 343–346.

MAGALETTI, E., R. URBANI, P. SIST, C. R. FERRARI, AND A. M. CICERO. 2004. Abundance and chemical characterization of extracellular carbohydrates released by the marine diatom *Cylindrotheca fusiformis* under N- and P-limitation. Eur. J. Phycol. **39:** 133–142.

MEON, B., AND F. JÜTTNER. 1999. Concentrations and dynamics of free mono- and oligosaccharides in a shallow eutrophic lake measured by thermospray mass spectroscopy. Aquat. Microb. Ecol. **16:** 281–293.

MIDDELBURG, J. J., C. BARRANGUET, H. T. S. BOSCHKER, P. M. J. HERMAN, T. MOENS, AND C. H. R. HEIP. 2000. The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labelling study. Limnol. Oceanogr. **45:** 1224–1234.

MILLER, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. **31:** 426–428.

MORAN, M. A., W. M. JR. SHELDON, AND R. G. ZEPP. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnol. Oceanogr. **45:** 1254–1264.

ORVAIN, F., R. GALOIS, C. BARNARD, A. SYLVESTRE, G. BLANCHARD, AND P.-G. SAURIAU. 2003. Carbohydrate production in relation to microphytobenthic biofilm development: An integrated approach in a tidal mesocosm. Microb. Ecol. **45:** 237–251.

OSBORN, A. M., E. R. B. MOORE, AND K. N. TIMMIS. 2000. An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. Environ. Microbiol. **2:** 39–50.

PERKINS, R. G., C. HONEYWILL, M. CONSALVEY, H. A. AUSTIN, T. J. TOLHURST, AND D. M. PATERSON. 2003. Changes in microphytobenthic chlorophyll *a* and EPS resulting from sediment compaction due to de-watering: Opposing patterns in concentration and content. Cont. Shelf Res. **23:** 575–586.

PINHASSI, J., F. AZAM, J. HEMPHÄLÄ, R. A. LONG, J. MARTINEZ, U. L. ZWEIFEL, AND Å. HAGSTRÖM. 1999. Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquat. Microb. Ecol. **17:** 13–26.

ROMANÍ, A. M., A. GIORGI, V. ACUÑA, AND S. SABATER. 2004. The influence of substratum type and nutrient supply on biofilm organic matter utilization in streams. Limnol. Oceanogr. **49:** 1713–1721.

RUDDY, G., C. M. TURLEY, AND T. E. R. JONES. 1998. Ecological interaction and sediment transport on an intertidal mudflat, I: Evidence for a biologically mediated sediment-water interface. Geological Society, London, Special Publications, **139:** 135–148.

SIMON, M., H-P. GROSSART, B. SCHWEITZER, AND H. PLOUG. 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquat. Microb. Ecol. **28:** 175–211.

SMITH, D. J., AND G.J.C. UNDERWOOD. 1998. Exopolymer production by intertidal epipelic diatoms. Limnol. Oceanogr. **43:** 1578–1591.

STAATS, N., L. J. STAL, B. DE WINDER, AND L. R. MUR. 2000. Oxygenic photosynthesis as driving process in exopolysaccharide production of benthic diatoms. Mar. Ecol. Prog. Ser. **193:** 261–269.

STAL, L. J. 2003. Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. Geomicrobiol. J. **20:** 463–478.

———, H. VAN GEMERDEN, AND W. E. KRUMBEIN. 1984. The simultaneous assay of chlorophyll and bacteriochlorophyll in natural microbial communities. J. Microbiol. Methods **2:** 295–306.

TAYLOR, I. S., D. M. PATERSON, AND A. MEHLERT. 1999. The quantitative variability and monosaccharide composition of sediment carbohydrates associated with intertidal diatom assemblages. Biogeochemistry **45:** 303–327.

UNDERWOOD, G. J. C. 2002. Adaptations of tropical marine microphytobenthic assemblages along a gradient of light and nutrient availability in Suva Lagoon, Fiji. Eur. J. Phycol. **37:** 449–462.

———, BOULCOTT, C. A. RAINES, AND K. WALDRON. 2004. Environmental effects on exopolymer production by marine benthic diatoms—dynamics, changes in composition and pathways of production. J. Phycol. **40:** 293–304.

———, AND J. KROMKAMP. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. Adv. Ecol. Res. **29:** 93–153.

———, AND D. M. PATERSON. 2003. The importance of extracellular carbohydrate production by marine epipelic diatoms. Adv. Bot. Res. **40:** 184–240.

———, D. M. PATERSON, AND R. J. PARKES, 1995. The measurement of microbial carbohydrate exopolymers from intertidal sediments, Limnol. Oceanogr. **40:** 1243–1253.

———, R. G. PERKINS, M. C. CONSALVEY, A. R. M. HANLON, K. OXBOROUGH, N. R. BAKER, AND D. M. PATERSON. 2005. Patterns in microphytobenthic primary productivity: Species-specific variation in migratory rhythms and photosynthetic efficiency in mixed-species biofilms. Limnol. Oceanogr. **50:** 755–767.

———, AND D. J. SMITH. 1998*a*. Predicting epipelic diatom exopolymer concentrations in intertidal sediments from sediment chl. *a*. Microb. Ecol. **35:** 116–125.

———, AND ———. 1998*b*. In situ measurements of exopolymer production by intertidal epipelic diatom-dominated biofilms in the Humber Estuary. Geological Society, London. Special Publications **139:** 125–134.

VAN DUYL, F. C., B. DE WINDER, A. J. KOP, AND U. WOLLENZIEN. 1999. Tidal coupling between carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats. Mar. Ecol. Prog. Ser. **191:** 19–32.

———, ———, ———, AND ———. 2000. Consequences of diatom mat erosion for carbohydrate concentrations and heterotrophic bacterial activities in intertidal sediments of the Ems-Dollard Estuary. Cont. Shelf Res. **20:** 1335–1349.

WUSTMAN, B. A., M. R. GRETZ, AND K. D. HOAGLAND, 1997. Extracellular matrix assembly in diatoms (Bacillariophyceae), I: A model of adhesives based on chemical characterisation and localisation of polysaccharides from the marine diatom *Achnanthes longipes* and other diatoms. Plant Physiol. **113:** 1059–1069.

ZAR, J. H. 1999. Biostatistical analysis, 4th ed. Prentice-Hall.

ZHOU, J., K. MOPPER, AND U. PASSOW. 1998. The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. Limnol. Oceanogr. **43:** 1860–1871.

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